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EVALUATION OF THE ANTIBACTERIAL ACTIVITY OF ARABICA COLD BREW AND ARABICA SPENT COFFEE GROUNDS ON STREPTOCOCCUS MITIS

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ABSTRACT

Spent coffee grounds or coffee waste pose a growing environmental problem as it can harm the ecosystem by releasing greenhouse gases into the atmosphere during their decomposition process [1]. On the other hand, spent coffee ground extracts have been shown to contain polyphenols, trigonelline, phenolic acids, and melanoidins which show strong antibacterial activity toward Gram+ bacteria and yeast [2]. Given its activity towards Gram+ bacteria, this study seeks to find an alternative use of spent coffee grounds, aiming to mitigate their environmental impact. This research focuses on the investigation on whether arabica spent coffee grounds could be used as a natural alternative to iodine mouthwash solution, a commonly used product to kill dental plaque bacteria. Arabica coffee extract was prepared by a 24-hour cold brew procedure then spent coffee grounds extract was produced from the Arabica coffee waste. To obtain results, the antibacterial activity of both spent coffee grounds and coffee on *S. mitis* was analyzed using the well diffusion test and inhibition zones were measured and compared. The caffeine contents of the Arabica coffee grounds were quantified using HPLC. From our quantitative analysis, the spent arabica coffee grounds collected from the cold brew procedure consist of less caffeine, compared to that of the cold brew, but still showed antibacterial activity towards *S. mitis*. The inhibition zone of the spent coffee grounds was significantly lower than cold brew coffee but still comparable to iodine mouthwash solution. Our novel findings indicate that spent coffee grounds can be used as a natural antibacterial and an alternative to povidone-iodine mouthwash which leads to reduction of environmental harm from coffee waste.

KEYWORDS: Streptococcus Mitis, Spent Coffee Grounds, Antibacterial Activity, Arabica Cold Brew, Caffeine Activity, Iodine Mouthwash

INTRODUCTION

Each year, around 23 million tons of spent coffee grounds are produced (Joshi, 2023). Each ton of discarded spent coffee grounds can emit as much as 340 cubic meters of methane (Engel, 2022). To reduce the impact of spent coffee grounds on the environment, spent coffee grounds can be utilized to produce value-added products. This study intends to highlight the utilization of spent coffee grounds in healthcare setting specifically.

Although *S. mitis* is generally considered as low pathogenic bacteria, it has a significant role in invasive infections especially those with poor oral care (Colomba et al., 2023), when travel to other organs from oral cavity, it can cause invasive diseases such as endocarditis, enteritis, and meningitis. Also, it is mostly found that the only predisposing factors can be identified in the poor oral hygiene of the patient and *S. mitis* is described as a major pathogen involved in the destruction of childhood dental enamel (Colomba et al., 2023). According to a recent study, Arabica spent coffee grounds as well as Robusta spent coffee grounds showed an inhibitory activity against *S. aureus* and *E. coli* at coffee waste concentrations of 1.0mg/mL posing as a natural alternative to povidone-iodione mouthwash (Díaz-Hernández et al., 2022). This paper is intended to study the potential usage of cold brew and spent coffee grounds brew to be utilized as a natural antibacterial and an alternative to povidone-iodine mouthwash, which will help to reduce coffee waste.

MATERIALS AND METHODS

Aceh Gayo Arabica coffee beans was bought from Nagadi Coffee and grounded coarsely using a home coffee grinder. Cold brew method was used in which the coffee was brewed in 2-8oC for 24 hours in water. According to the New York Times, cold brew ratio is 1 ground coffee: 10 ml water. Since previous studies never used cold brew, a maximum concentration of 200 mg/ml was chosen (New York Times, 2023). Initial stock solution of cold brew 200mg/ml was prepared by mixing 20g of ground coffee with 100ml distilled water. This cold brew was then filtered using coffee filter paper to obtain the 20mg/ml cold brew stock solution and the spent coffee ground (SCG). Then a serial dilution of the cold brew was done to prepare these concentrations: 100mg/ml, 75mg/ml, 50mg/ml and 25 mg/ml, using distilled water. To make 200mg/ml SCG brew stock solution, 20g of SCG was cold brewed in 100ml distilled water Another set of serial dilutions of SCG brew, using distilled water, was prepared: 200 mg/ml, 100mg/ml, 75mg/ml, 50mg/ml and 25 mg/ml.

Commercial 1% povidone-iodine mouthwash (mouthwash) contains 10mg/ml povidone iodine. From the mouthwash bottle, a serial dilution was done to prepare the following concentrations: 10mg/ml, 5mg/ml, 2.5mg/ml, 1mg/ml and 0.5mg/ml, using distilled water.

The *Streptococcus mitis* (NCIMB 13770) was supplied from PT. Embrio Biotekindo (MBrio) and cultured following MBrio's culture certificate in Sheep Blood Agar (SBA). Aseptically, 0.1 ml of TSB media was added into the bacteria strain ampule. Sterile cotton swab was used to homogenize the mixture then the mixture was inoculated on the SBA petri dish. This petri dish was put into an anaerobic jar and incubated in 37oC for 24 hours. One ose of the inoculate was mixed with 10 ml of 0.9% NaCl. The absorbance of this bacterial solution was measured using UV/visible spectrophotometer at 580nm to prove its concentration with %T of 25%, following USP <81> on inhibition testing. After testing, 1ml of inoculum was added into 100ml of SBA to make SBA+inoculum culture stock.

The susceptibility of *S. mitis* to cold brew, SCG brew and oral mouthwash were assessed by using the well diffusion test. A stainless-steel cork borer with inner diameter of 3.6 mm was used to make the wells. S. mitis agar dish was prepared by adding 4ml of SBA+inoculum onto a layer of solidified 21ml SBA. After bacteria and SBA solidify, 4 wells were made on each bacteria agar (one well mouthwash were added into each well. One bacteria agar had one type of concentration, so each agar had 4 wells of the same concentration of a sample solution. The above tests were performed in duplo for each sample. A negative control plate was prepared. All agar plates were incubated in 37oC for 24 hours. Minimum inhibitory concentrations (MIC) of each sample were determined by measuring the diameter of inhibition.

Caffeine content analysis was carried out at PT Saraswanti Indo Genentech using HPLC-PDA. Caffeine contents were measured for the following coffee samples: cold brew 200mg/ml and 25mg/ml, and SCG brew 200mg/ml and 25 mg/ml.

Statistical analysis was performed using paired t-test on Excel software on the well diffusion test result of coffee extract. The trial was done in duplo so each sample had 8 wells. The inhibition zone of the SCG brew and cold brew solution were reported as the mean (with standard deviation). The significance level was set at $p < 0.05$ and $p < 0.05$ was considered to be different statistically at significant degree.

Concentration (mg/ml)	Diameter mean (mm) ± SD		Mouthwash concentration (mg/ml)	Diameter mean (mm) ± SD
	Spent Coffee	Cold Brew Solution		
25	6.59±0.44	8.88±0.33	0.5	8.86±0.54
50	7.62±0.26	9.26±0.26	1.0	7.61±0.25
75	7.79±0.18	9.31±0.36	2.5	7.84±0.18
100	8.32±0.19	9.52±0.17	5.0	7.84±0.15
200	9.36±0.39	10.65±0.47	10.0	9.94±0.65

Table 1. Inhibition zones of spent coffee ground brew solution, cold brew solution, and mouthwash solution against *Streptococcus mitis* (NCIMB 13770) at 24 h

Inhibition zone of cold brew solution showed higher results for all concentrations compared to SCG brew solution. Evaluation of paired t-test with $\alpha=0.05$ results the inhibition of SCG brew solution on all concentrations show a significant difference ($p < 0.05$) compared to the cold brew solution. Inhibition is the lowest at concentration of 25 mg/ml for both cold brew and SCG brew.

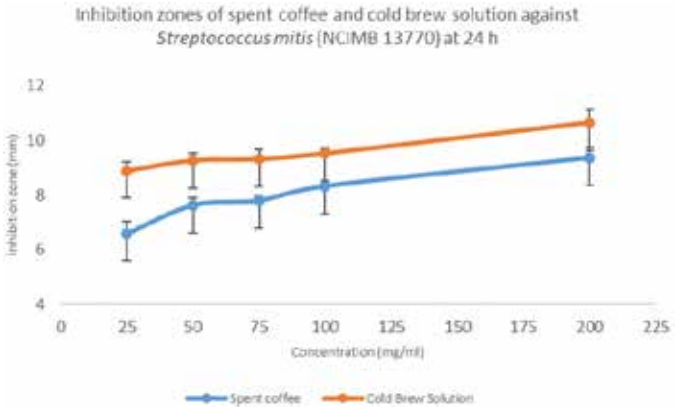

Figure 1. Inhibition zone profile of cold brew solution and spent coffee ground brew solution against *Streptococcus mitis* at 24 h.

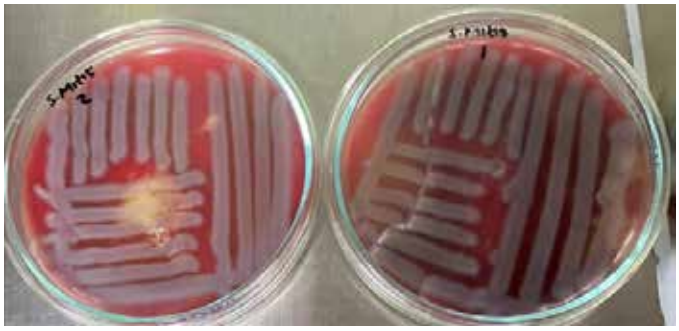

Figure 2. Profile of *Streptococcus mitis* growth for inoculum

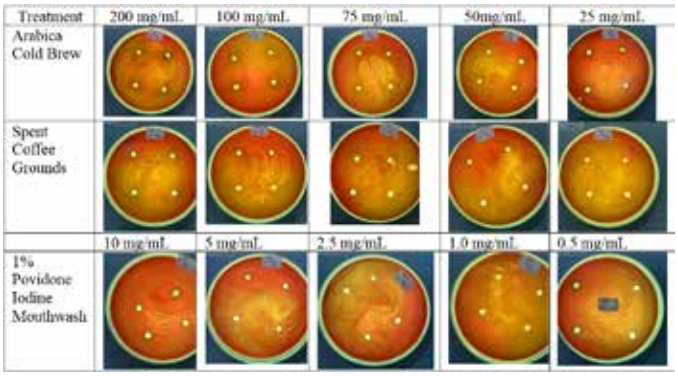

Figure 3. The susceptibility of *Streptococcus mitis* to coffee extract and mouthwash solution.

The well diffusion test was used to determine the MIC. Agar plates were treated with bacteria and (a) 25 mg/mL, (b) 50 mg/mL, (c) 75 mg/mL, (d) 100 mg/mL, and (e) 200 mg/mL cold brew solution or spent coffee ground brew solution. Povidone Iodine Mouthwash solution as comparator used in (a) 0.5 mg/mL, (b) 1.0 mg/mL, (c) 2.5 mg/mL, (d) 5.0 mg/mL, and (e) 10.0 mg/mL.

Concentration (mg/ml)	Caffeine Content (mg/ml)	
	Spent Coffee	Cold Brew Solution
200	2.142±0.0014	0,722±0.0007
25	0,106±0.0002	0,097±0.0005

Table 2. Caffeine concentration of coffee extract

DISCUSSION

In this research, the antibacterial inhibitory effects of Arabica coffee cold brew, Arabica spent ground coffee brew and oral mouthwash at various concentrations on the growth of *S. mitis* were determined using the well diffusion method. Antibacterial inhibitory effects were indicated with the existence of a clear zone around the well. Due to limited resources of extraction equipment, home coffee grinder and coffee filter were used and the simplest method to gain the most caffeine extract was chosen, which is cold brewing (Martin, 2016).

The results showed in Table 1 shows that all samples of Arabica coffee cold brew, Arabica spent ground coffee brew and oral mouthwash were able to inhibit the growth of *S. mitis* at different levels of MIC. As seen on Fig 1, we can see that there is a significant difference between the inhibition zones of cold brew than those of spent coffee ground.

HPLC-PDA analysis (Table 2) revealed that Arabica cold brew at 200mg/mL has a higher concentration compared to SGC brew while at 25mg/ml concentrations, cold brew and SCG brew had almost the same caffeine content. This shows that caffeine is highly likely not the main component for antibacterial activity. According to Gaul & Donegan, the components of coffee that allows it to contain antibacterial activity are trigonelline, chlorhexidine and fluoride. However, since resources were limited, these other components in the coffee solutions could not be determined and only caffeine concentrations were measured.

A study comparing the antibacterial components of green coffee beans, roasted coffee and spent ground coffee showed that spent ground coffee has higher content of total phenolic compounds compared to roasted coffee ground (Díaz-Hernández et al., 2022). Being a by-product of coffee, spent coffee ground brew is still possible to have a higher content in certain components compared to cold brew. As correlated with the graph and previous studies, caffeine may not be the main component that acts as an antibacterial agent (Okabe et al., 2004). Despite this study's limitation, spent coffee grounds still had activity against *Streptococcus mitis* showing that it does have a potential to be used into beneficial agents instead of harmful waste. Moreover, cold brew consistently showed a higher zone of inhibition compared to spent coffee grounds which strongly suggests that there is a certain component in normal cold brew that enhances the antibacterial activity which could be the trigonelline, chlorhexidine and fluoride. (Okabe et al., 2004)

Looking at Table 1, overall, oral mouthwash seemed to have higher MIC but there is not a correlation can be drawn between mouthwash's different concentration with its MIC. However, data also showed that mouthwash's MIC values at 10mg/ml were comparable to cold brew 100mg/ml and SCG brew 200 mg/ml. Mouthwash (1% povidone iodine) is usually used by swishing 15ml of the solution in the mouth for 30 seconds without any dilution, so the normal concentration used is 10 mg/ml. All MIC were measured after 24 hours of incubation while usually either mouthwash or coffee are kept in the oral cavity for 24 hours. To better mimic real situation, further study needs to be done when the time of contact is 30-60 seconds.

CONCLUSION

In summary, this study revealed a novel use of coffee and spent coffee ground, which is an antibacterial activity against *Streptococcus mitis* with antimicrobial activity and that antimicrobial activity was comparable to that of marketed mouthwash and gargle 1%. The next step is to investigate on the properties of spent coffee grounds that allow for antibacterial activity so the precise active biomolecule can be extracted properly. Moreover, a further action towards designing a commercial product is to study different preparations of spent coffee grounds to ensure longer stability and homogeneity of its contents, as well as make it more consumer friendly. Despite this, this paper concludes that the usage of spent coffee ground as mouthwash could be a potential alternative to reduce coffee waste which leads to reduction of carbon emission.

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